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Ethnic Differences in Insulin Granule Exocytosis
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Type 2 diabetes (T2D) is diagnosed based on high fasting plasma glucose (FPG) or high glucose at the two-hour time point of an oral glucose tolerance test (OGTT). However, clinical data show that this may not be appropriate for all ethnic groups. For example, Koreans often have high glucose one hour into the OGTT but normal FPG and two-hour glucose. We have developed a comprehensive model of the pathogenesis of T2D, based on the model of Topp et al (J. Theor Biol. 2000), modified to include a subsystem for exocytosis, and have applied it to simulate OGTTs. The model suggests that the Korean OGTT pattern results a defect in early (first-phase) insulin secretion and a delay in late (second phase) secretion. This may contribute to the high prevalence of T2D in Korea and points to the danger of under-diagnosis using the standard criteria.
In contrast, African Americans typically exhibit strong first-phase insulin secretion relative to Whites and second phase insulin secretion similar to that of Whites. This group therefore also has normal FPG and two-hour glucose during OGTT, which again can result in under-diagnosis of their high risk for T2D. The simulations for this case suggest that the strong first phase results from a large readily releasable pool of insulin granules but that the diabetes risk results from trafficking of reserve vesicles to the plasma membrane that is more susceptible to deterioration under the stress of insulin resistance.
We conclude that a detailed analysis of insulin secretion dynamics is necessary to properly interpret OGTT results for ethnically diverse populations.

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Quantitative Imaging of the Exocytosis Machinery Assembly
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The assembly of the secretory machinery is a poorly understood prerequisite for regulated exocytosis. Current models propose that the arriving vesicle docks at the plasma membrane by binding to either raft-like clusters (nanodomains) of SNARE proteins or to structural proteins such as RIM1, in both cases implying at least partial assembly of the secretory machinery prior to docking. In contrast, we recently showed that docking coincides with and requires recruitment of syntaxin and munc18 into nanodomains at the docking site, suggesting assembly after docking. Here we extend on this work and present live cell imaging-based quantification for many exocytosis proteins (including syntaxin, SNAP25, Munc18, Munc13, Rab3+27, Rabphilin, Granuphilin, RIM1, CaV1.2, EPAC, NSF, alfaSNAP; all tagged with EGFP) at the insulin granule release site during docking, priming and exocytosis. We find that the Rab3 interacting protein RIM1 was the only protein enriched at docking sites prior to vesicle tethering and docking. Further recruitment of RIM1 to the docking site occurred during granule maturation into the releasable pool (priming), suggesting roles in both docking and priming. None of the other proteins were present before granule arrival, but these were instead recruited during docking or even later during priming. Granules that successfully docked carried Rab3 and Rabphilin, whereas those that only temporarily tethered did not. In contrast, Rab27 and its effector Granuphilin were present on both types of granules. We conclude that sites enriched in RIM1 at the plasma membrane may facilitate docking by weakly tethering the incoming granule through interaction with rab3/rabphilin. Successful docking requires acute clustering of syntaxin/munc18, and we propose that this cluster then nucleates assembly of the exocytosis machinery.

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Fusion Properties of Gliotransmitter Vesicles in Cultured Astrocytes
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Astrocytes actively participate in brain signalling by releasing a plethora of gliotransmitters which can modulate synaptic transmission. Vesicle-based mechanisms mediate the release of gliotransmitters. However, the anatomy and nature of exocytotic vesicle interaction with the plasma membrane is unclear. Using STED and SIM super-resolution microscopies, we studied the morphology of distinct gliotransmitter vesicles, whereas the interaction between a single vesicle with the plasma membrane was monitored by measurements of membrane capacitance (Cm), a parameter linearly related to the surface area of the plasma membrane. Immunolabelling of vesicles containing D-serine, glutamate, atrial natriuretic peptide (ANP) and brain derived neurotrophic factor (BDNF) yielded their diameter to be ~70 nm, whereas ATP was found in larger vesicles (~200 nm diameter). Cell-attached measurements have shown the predominant reversible unitary exocytotic events exhibiting transient fusion were found in two different populations, the first corresponding to smaller vesicles with diameters around 70 nm and the second corresponding to bigger vesicles with the median at 200 nm, consistent with the STED and SIM measurements. Upon stimulation with ATP, which increases intracellular Ca^{2+} concentration, the smaller vesicles persisted in transient fusion, whereas the bigger vesicles proceeded to full fusion. This was interpreted previously to be due to different SNARE protein densities in different sized vesicles and was tested here by astrocyte treatment with botulinum neurotoxin D (BotD), which significantly reduced the occurrence of unitary exocytotic events for both vesicle types. Additionally, when we expressed dominant-negative SNARE in astrocytes, the fusion-pore diameter was narrower in both vesicle types. Taken together, this work shows that vesicle content discharge is modulated by vesicle size, however independently of the functional integrity of the SNARE proteins, indicating that SNARE-dependent mechanisms determining vesicle merger with the plasmalemma are not strictly part of those modulating fusion-pore geometry.

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Probing the Interaction Between Synaptotagmin-1 and SNARES using Mutations in SNAP-25
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Synaptotagmin-1 (syt-1) is the main Ca^{2+} sensor for triggering fast, synchronous neurotransmitter release through exocytosis of synaptic vesicles at the presynapse. The SNARE-complex (consisting of SNAP-25, VAMP-2/synaptobrevin-2 and syntaxin-1), which assembles between vesicle and plasma membrane, is required for membrane fusion. The precise mode of interaction between syntaptotagmin-1 and the SNARE-complex remains unresolved. Measurements in syt-1 null cells revealed an 'unclamping' phenotype (increased mini rate) as well as the absence of fast synchronous release. Whether both phenotypes depend on SNARE-interaction is unclear.
In order to address the role of the syt-1:SNARE-interaction during synaptic release we introduced three set of SNAP-25 mutations into snap-25 null autaptic neurons, which by different laboratories have been reported to impair syt-1:SNARE interactions. These mutations are situated in the C-terminal end, around the middle of the bundle, and towards the N-terminal end (Mohrmann et al., 2013). Hippocampal autaptic cultures displayed significantly increased spontaneous release rates in all three SNAP-25 mutations in comparison to wildtype rescue experiments. In addition, the N-terminal and central mutations nearly abolish evoked synchronous release, whereas the C-terminal SNAP-25 mutation did not display a difference in EPSC size compared to the SNAP-25 wildtype. Different analyses overall showed that the C-terminal mutation resembles the wild-type as far as evoked release is concerned, whereas the N-terminal and central mutations have severe consequences for evoked release. Our experiments show that the correct interplay between Syt-1 and SNAP-25 is crucial for properly regulated release at the synapse, and that this interplay might be differently organized for evoked and spontaneous release.
Mohrmann et al., 2013, J. Neurosci. 33(36):14417-144430.

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Induction of Hippocampal Synapses on Functionalized Micropatterns
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We developed a novel presynaptic neuronal preparation in culture. Using microstructured coverslips functionalized with protein domains of synaptic cell adhesion molecules (SCAMs) as artificial postsynapse we are able to induce hippocampal neurons to form hybrid synapses, i.e. purely presynaptic structures 'en face' directly onto the coverslip.
Axons growing over such micropatterns, functionalized with SCAM domains by click chemistry, form large flat varicosities. 4Pi microscopy revealed the presence of several presynaptic markers like the synaptic vesicle protein Synaptophysin1 or the active zone scaffold proteins RIM1/2, while postsynaptic staining against e.g. PSD 95, HOMER and the AMPA receptor was absent. Serial section transmission electron microscopy (TEM) as well as focused ion beam scanning electron microscopy (FIB-SEM) confirmed that these varicosities harbor a few hundred synaptic vesicles in several clusters near and at the bottom membrane and show typical active zone hallmarks at the bottom